Impact of Restoration Practices on Mycorrhizal Inoculum Potential in a Semi-Arid Riparian Ecosystem

by

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ABSTRACT

Mycorrhizal fungi form symbiotic relationships with plant roots, increasing nutrient and water availability to plants and improving soil stability. Mechanical disturbance of soil has been found to reduce mycorrhizal inoculum in soils, but findings have been inconsistent. To examine the impact of restoration practices on riparian mycorrhizal inoculum potential, soil samples were collected at the Tres Rios Ecosystem Restoration and Flood Control Project located at the confluence of the Salt, Gila, and Agua Fria rivers in central Arizona. The project involved the mechanical removal of invasive *Tamarix* spp.(tamarisk, salt cedar) and grading prior to revegetation. Soil samples were collected from three stages of restoration: pre-restoration, soil banks with chipped vegetation, and in areas that had been graded in preparation for revegetation. Bioassay plants were grown in the soil samples and roots analyzed for arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) infection percentages. Vegetations measurements were also taken for woody vegetation at the site. The mean number of AM and EM fungal propagules did not differ between the three treatment area, but inoculum levels did differ between AM and EM fungi with AM fungal propagules detected at moderate levels and EM fungi at very low levels. These differences may have been related to availability of host plants since AM fungi form associations with a variety of desert riparian forbs and grasses and EM fungi only form associations with *Populus* spp. and *Salix* spp. which were present at the site but at low density and canopy cover. Prior studies have also found that EM fungi may be more affected by tamarisk

invasions than AM fungi. Our results were similar to other restoration projects for AM fungi suggesting that it may not be necessary to add AM fungi to soil prior to planting native vegetation because of the moderate presence of AM fungi even in soils dominated by tamarisk and exposed to soil disturbance during the restoration process. In contrast when planting trees that form EM associations, it may be beneficial to augment soil with EM fungi collected from riparian areas or to pre-inoculate plants prior to planting.

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CHAPTER 1

INTRODUCTION

Restoration efforts have increased in the riparian areas of rivers in the southwestern U.S. since the 1990's (Shah et al. 2007). Dams, diversions, agriculture, mining, urbanization, and overgrazing of rangelands have resulted in altered hydrology, elevated levels of salts, nutrients, sediment, pollution (Shah et al. 2007) and invasion by non-native plants such as *Tamarix* spp. (tamarisk, salt cedar) in these ecosystems (Shafroth et al. 2005). Restoration practices that mechanically disturb soil may impact soil microbial communities, including mycorrhizal fungi (Schnoor et al. 2011; Urcelay et al. 2009). The purpose of this study was to examine the impact of the mechanical removal of tamarisk and re-grading of soil on mycorrhizal inoculum potential in a semi-arid, riparian ecosystem undergoing restoration. The site selected for the study was the Tres Rios Ecosystem Restoration and Flood Control Project, located at the confluence of the Salt, Gila, and Agua Fria rivers in southwestern Phoenix, Arizona. At the time of the study, tamarisk was being mechanically removed, the river re-channeled, and the banks graded in preparation for revegetation.

Mycorrhizal fungi play a functional role in riparian ecosystems by increasing nutrient availability to plants, improving soil stability and improving drought tolerance of plants with which they form associations (Stutz et al. 2009). Mycorrhizal fungi are soil fungi that form symbiotic relationships with plant roots. Approximately 80 % of terrestrial plant families form mycorrhizal associations (Trappe 1987). The fungi benefit from

the association by obtaining photosynthetically derived carbohydrates from the plant. Plants benefit by increased access to water and nutrients including phosphorus, nitrogen, copper, iron and zinc. Mycorrhizal fungi colonize plant roots and form external hyphae that extend into the soil. A portion of the water and nutrients obtained by the fungi is transferred to the plant (Smith and Read 1997; Govindarajula et al. 2005). Mycorrhizal associations have been found to alter plant community structure by enhancing the ability of plants with mycorrhizal associations to compete with non-mycorrhizal plants (Beauchamp et al. 2005).

Arbuscular mycorrhizae (AM), also known as vesicular arbuscular mycorrhizae (VAM), and ectomycorrhizae mycorrhizae (EM) are the two most common types of mycorrhizal associations in riparian ecosystems (Stutz et al. 2009). AM fungi are in the phylum Glomeromycota (Schussler et al. 2001). They colonize plant roots internally by forming arbuscules between the cell wall and plasma membrane of root cortex cells. EM fungi are found in the phyla of both Basidiomycota and Ascomycota (Smith and Read 1997). EM fungi colonize plant roots externally, forming a dense fungal sheath or mantle that covers the outside of the root tips, and internally forming a Hartig net of hyphae that grows within the root cortex.

Mycorrhizal fungi propagate from spores, pieces of extra radical hyphae and colonized root fragments (Klironomos and Hart, 2002). Activities of soil invertebrates, wind erosion of soil (Warner et al. 1987) and mammals (Allen 1987) aid in the dispersal of AM fungi. EM fungal spores

disperse primarily by wind although mammal dispersal is important for some species (Johnson 1996).

Many desert riparian forbs and grasses have been found to form AM associations (Kennedy et al. 2002; Beauchamp et al. 2006). *Populus* spp.(cottonwood) and *Salix* spp. (willow) trees, which are dominant trees in the Sonoran Desert floodplains, and part of the revegetation plans for this site, have been found to form tripartite associations with both AM and EM fungi (Lodge 1989; Lodge and Wentworth 1990; Sasaki et al. 2001). Tamarisk has not been found to form associations with EM fungi. Low levels of colonization by AM fungi have been detected as well as colonization by dark septate endophytes (Beauchamp et al. 2005; Titus et al. 2002).

The presence of tamarisk has been found to disrupt cottonwood mycorrhizal associations (Meinhardt and Gehring, 2012). In the field studies, Meinhardt and Gehring (2012) found that cottonwoods with tamarisk neighbors had reduced AM and EM colonization and that there was reduced EM propagule abundance in the soil beneath the tamarisk plants. In a greenhouse study, they found reduced EM but not AM colonization in cottonwoods grown with tamarisk. The cottonwoods had benefited by EM colonization as evidenced by increased shoot biomass when grown with a conspecific but not when grown with tamarisk. The reduction in EM abundance may have been due to the alteration in soil chemical properties associated with tamarisk; increased NO_3^- levels and electrical conductivity were found in soil near tamarisk plants in the field.

Findings regarding the impact of mechanical disturbance of soil on mycorrhizal communities have been mixed. Urcelay et al. (2009) examined the impact of plant functional type (shrub, perennial forb, annual forb and graminoid) removal on AM communities. Five months after initial removal, there was a reduction in AM fungal colonization in all plant removal treatments and a mechanically disturbed control plot in comparison to the undisturbed control plots. However, the effects were no longer present after 17 months. Schnoor et al. (2011) examined the impact of mechanical disturbance (i.e. plowing) on AM fungal communities within a semi-natural grassland that was being restored. The disturbance significantly reduced Glomeromycota phylotype richness and changed the AM fungal community composition. Richter et al. (2002) assessed AM inoculum potential in grasslands and abandoned agricultural fields in riparian areas in southeastern Arizona. They found that mean infection percentages were higher in an abandoned agricultural field in which *Salsola tragus* L. was grown for cattle feed and promoted by disking every 3 to 4 years as compared to a neighboring grassland. Studies in other agricultural settings have found conventional tillage practices reduce mycorrhizal spore levels in comparison to reduced or no-till conditions (Galvez et al. 2001; Borie et al. 2006; Li et al. 2007; Celik et al. 2011).

The current study presented a unique opportunity to collect soil samples during three stages of an ongoing restoration project: pre-restoration, after tamarisk had been mechanically removed and soil banked, and after grading in preparation for planting of native species, providing an

opportunity to examine the impact of vegetation removal and soil movement on propagules/inoculum (spores, actively growing hyphae, and dormant pieces of hyphae) of mycorrhizal fungi. Because of previous work on the impact of disturbance on mycorrhizal fungi, it was hypothesized that soil from the soil bank and graded conditions would have a reduced level of inoculum in comparison to the pre-restoration condition due to disruption of hyphal networks from the tamarisk removal and soil movement, with inoculum levels lowest in the graded condition.

The results of this study should contribute to an understanding of the resilience of riparian mycorrhizal fungi to mechanical disturbance and provide implications for riparian restoration practice by determining if inoculation with mycorrhizal fungi is needed during revegetation.

CHAPTER 2

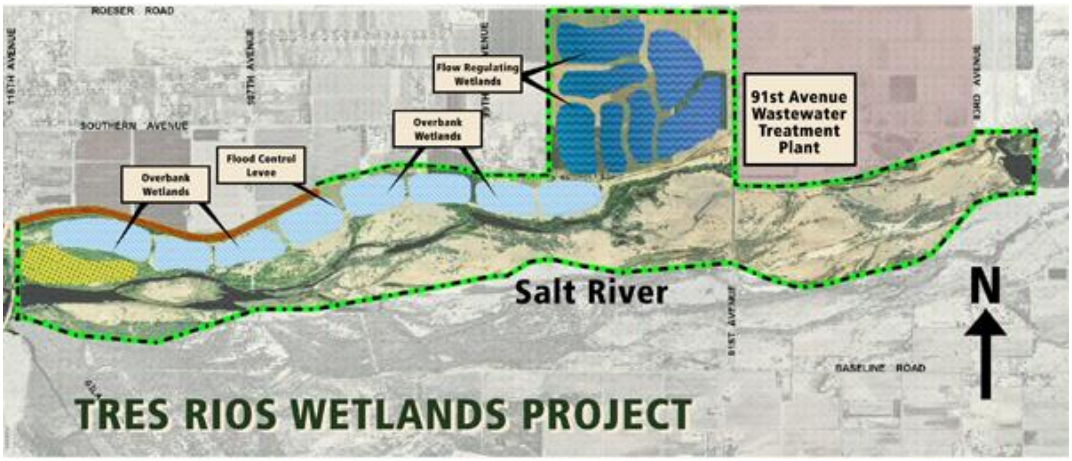
METHODS

Study Area

Soil and root samples and vegetation measurements for this study were taken at the Tres Rios Ecosystem Restoration and Flood Control Project (Figure 1) in southwestern Phoenix, Arizona (33° 23' 11" N, 112° 18' 41" W). The overall project area encompassed 6.07 km^2 and has consisted of three phases (1) two flood protection levees, completed June 2008, (2) emergent wetlands, completed July 2010 and an effluent wastewater pump station, completed July 2012 and (3) riparian corridors and open water marsh areas to replace existing non-native salt cedar in the river, completed July 2012 (City of Phoenix 2012). The soil and root samples and vegetation measurements for this study were collected from the riparian corridor that is part of Phase 3 of the restoration.

Effective mechanical removal of tamarisk requires the removal of root crowns in addition to above ground material to avoid re-sprouting (Shafroth et al. 2005). At the project site, tamarisk was being mechanically removed with excavators. Soil was removed to a depth of 46 cm to ensure removal of roots and stumps. Cottonwood and willow trees were left standing when possible. After removal, the tamarisk was chipped. Large tracts of soil which included the chipped tamarisk were collected in soil banks. The river was re-channeled and the soil graded in preparation for planting of cottonwood and willow trees and seeding of other native vegetation (Newman, personal communication).

Figure 1. Map of the Tres Rios Ecosystem Restoration and Flood Control Project (City of Phoenix 2012).



Soil Sampling

Soil samples were collected from three site treatments: (1) pre-restoration, an undisturbed site with both tamarisk and native vegetation (collected April 2011), (2) soil banks which included vegetation that had been mechanically removed and chipped (collected April 2011), and (3) an area that had been graded in preparation for planting of native species (collected June 2011). The timing of sample collection was determined by availability of the sites. Within each treatment, 25 soil samples were collected using a random walk method. Starting at approximately the center of the site, a random number table was used to identify the direction of the walk (1 to 12 watch numbers) and the number of paces (up to 30). Soil was collected up to a 15 cm depth, placed in sealed 2 quart plastic bags, and kept in a cooler until transported to the laboratory where the samples were then kept under refrigeration.

Soil Analyses

Three soil samples were selected randomly for analysis from each of the three treatments (pre-restoration, soil bank, and graded). The following soil qualities were assessed: pH, electrical conductivity, dry color, percent coarse fragments and texture.

Each soil sample was sieved with a 2 mm sieve. Coarse fragments (> 2mm) were weighed and removed from the soil samples. The remaining soil was then weighed and percent coarse fragments calculated. The samples were then split and all further analyses were completed in duplicate to ensure reliability of the results.

A Fisher Scientific Accumet Portable pH Meter, standardized with pH 4, pH 7, and pH 10 buffers was used to measure pH. Forty grams of air dried soil was placed in a beaker, 10 mL of distilled water was added and the solution was mixed well. After standing for 10 minutes, the suspension was swirled in the beaker and then the pH electrode was inserted into the suspension to read the pH.

An Oakton RD 232 Conductivity Meter was used to assess electrical conductivity. The meter was calibrated using the following concentrations of KCL: 84µ, 1413 µ, and 2070µ. Thirty grams of air dried soil was placed in a flask and enough distilled water was added to create a stable soil paste. The paste was allowed to sit for 30 to 60 minutes to permit saturation and was stirred at the end of the waiting period. A Buchner funnel was fitted with a stopper was placed into a 250 mL Erlenmeyer flask connected to a vacuum port. Filter paper was placed in the funnel and moistened with a small amount of distilled water. The soil paste was added to the funnel and allowed to drain with vacuum filtration until the paste cracked. The solution collected in the flask was poured into a test tube and the electrical conductivity measured.

To determine color, air dried samples were viewed under natural lighting conditions and compared with a Munsell Soil Color Chart.

To determine texture, 50 grams of soil was added to a stirring cup, the cup was filled half full with distilled water and 10 mL of sodium hexametaphosphate. The solution was stirred for 5 minutes with a mixing machine and quantitatively transferred to a settling cylinder. Distilled water

was added to fill the cylinder to the one liter mark and the solution was vigorously stirred in with up and down strokes. A hydrometer was placed in the liquid and after 40 seconds the hydrometer was read. The solution was then stirred and the readings repeated until they were within 0.5 grams of each other. The temperature was read for later temperature corrections. The solution was re-stirred and let stand for two hours then hydrometer and thermometer readings were taken. Hydrometer readings were corrected by adding 0.25 for each degree above 18° C. The readings did not have to be corrected for lower temperatures as there were no readings below 18°C.

A soil moisture correction factor was determined for each sample by weighing approximately 10 g of soil and placing it into a small tin. The samples were placed in an oven at 110°C for 24 hours. The samples were re-weighed after 30 minutes in a desiccator.

Vegetation Measurement

In the pre-restoration treatment area, vegetation measurements were taken using the Point-Quarter method (Brower et al. 1998). Measurements were taken from the soil sample points to the nearest woody species in the north, east, south, and west quadrants; distance to plant center and canopy intercept were recorded as well as species.

Mycorrhizal Root Colonization

The soil samples from the pre-restoration area were examined for presence of roots to assess colonization of vegetation at the sampling site prior to plant removal. Roots were collected from each sample and fixed in a 50% ethanol solution. They were cleared in 2.5% KOH and stained in Trypan

Blue utilizing the method of Koske and Gemma (1989). A modified grid-line intercept method, utilizing a dissecting microscope, was used to determine the percentage of roots colonized by AM and EM fungi (Giovanetti and Mosse 1980).

Mycorrhizal Inoculum Potential Bioassay

The bioassay host for AM fungi was *Zea mays var. saccharata* (sweet corn, cultivar: Bi-licious). Prior to planting, the corn seeds were surface sterilized in a 10% bleach solution for three minutes. One hundred-fifty nursery cone-tainers (Stuewe & Sons Inc., 3.8 cm × 21 cm) and 175 2.2 cm glass marbles were surface sterilized in 10% commercial bleach (>1 h). One marble was placed in the bottom of each cone-tainer before adding soil to prevent soil loss. A 125 mL sub-sample of soil from each of the 75 samples was mixed with 125 mL of sterilized sand and separated into two replicate cone-tainers. Three corn seeds were planted per cone-tainer and top watered three times per week. Approximately 25 mg of 20/0/20 slow release nitrogen fertilizer (Par Ex Professional Products, Stone Container Corp.) was added per cone-tainer when the corn plants were at the three leaf stage. The corn plants were harvested after 30 days of growth. The harvested corn roots were fixed in a 50% ethanol solution then cleared in 2.5% KOH and stained in 0.5%Trypan Blue utilizing the method of Koske and Gemma (1989). A modified grid-line intercept method, utilizing a dissecting microscope, was used to determine the percentage of roots colonized (Giovanetti and Mosse 1980).

The assay host for EM fungi was *Populus fremontii* (cottonwood). Cottonwood seeds were collected in April 2011 from trees along the riparian area of the Salt River approximately 22.5 km east of the study area. The cottonwood seeds were surface sterilized using a 10% bleach solution for 3 minutes, sown in flats containing silica sand that had been autoclaved at 121° C/ 15 psi for 1 hr, and covered with a thin layer of the sterilized sand. The flats were placed in a greenhouse under natural lighting and bottom watered continuously; liquid fertilizer 24/8/16 Nitrogen Phosphate Potash (Miracle-Gro) was added to the water weekly (2.5 mL added to 3.8 L of water per flat). The seedlings were thinned to three plants per cell after four weeks of growth.

Nursery cone-tainers (Stuewe & Sons Inc., 6.25 cm × 26 cm) were surface sterilized in 10% commercial bleach (>1 h) and lined with paper baking cups to prevent soil loss. A 225 mL sub-sample of soil from each of the 75 samples was mixed with 225 mL of sterilized sand and placed into the cone-tainers.

Cottonwood seedlings were transplanted into the pre-restoration and soil bank samples six weeks after germination and into the graded samples 9.5 weeks after germination; the time period difference was due to construction related delays in obtaining the graded samples. The seedlings were kept in a greenhouse under natural lighting. The soil in the cone-tainers was kept moist by bottom watering. The cottonwood seedlings were harvested after 30 days of growth in soil samples. The harvested cottonwood roots were fixed in a 50% ethanol solution. A modified grid-line intercept

method, utilizing a dissecting microscope, was used to determine the percentage of roots with ectomycorrhizal tips (Giovanetti and Mosse 1980).

Colonization of Cottonwoods and Willows

Due to low levels of EM colonization of bioassay host plants, roots were collected from living cottonwood and willow trees at the study site in April of 2012 to assess colonization levels. Roots were collected from ten cottonwood and ten willow trees. EM colonization was assessed using the same methods used to assess colonization in the bioassay plants.

Statistical Analyses

Soil pH and electrical conductivity mean and standard error of the mean (SE) values were calculated. Mean pH per condition (pre-restoration, soil bank, and graded) was computed by converting the values to concentration of hydrogen ions (10^{-pH}), calculating the mean, then converting back to pH ($-\log[H^+]$). The soil moisture correction factor was calculated by dividing dry soil weight by moist weight. Temperature and moisture corrected hydrometer readings were used for texture calculations. The 40 second hydrometer reading provided the value for grams of sand; the 2 hour hydrometer reading for grams of clay. The values were converted to percentages and percent silt obtained by subtraction. Soil textural class was obtained by utilizing a soil texture triangle.

Vegetation density (plants/ha), canopy cover (m^2/ha) and relative species dominance based on cover was calculated for woody species in the undisturbed condition using the procedures outlined by Brower et al. (1998).

AM bioassay (mean infection percentages) MIPs were arcsine transformed to meet assumptions of normality. Analysis of variance (ANOVA) was conducted using the arcsine values to compare the three conditions (pre-restoration, soil bank, graded). Mean, range and SE values were also calculated. The EM bioassay data and the AM/EM data from roots present in soil in the undisturbed condition were not analyzed using ANOVA. Assumptions of normality could not be met due to the high number of root samples with no colonization. Mean, range and SE of the MIP values were calculated for EM colonization of the willow and cottonwood tree roots.

To determine whether the bioassay plants colonized by EM fungi had been planted in soil that was taken at points near EM vegetation, the Fischer's Exact Test of Independence was performed.

Spreadsheet formulas using Microsoft Office Excel 2007 were used for all computations. Pre-set formulas provided by McDonald (2009) were used for the ANOVA and Fischer's Exact Test of Independence analyses.

CHAPTER 3

RESULTS

Differences in pH and EC values were observed between the different treatment areas (Table 1). The pH of each assessed soil samples fell in the slightly to moderately alkaline range per the Natural Resources Conservation Service categories (NRCS, 1993) with the highest pH values detected in soil samples collected in the pre-restoration treatment (range 7.99-8.43) and the lowest and most uniform values detected in soil collected from the graded area (range7.48-7.76). Electrical conductivity (EC) of the soil was between 0.04 mS/cm and 6.41 mS/cm with the EC of soil collected in the graded treatment higher than the EC detected in soil collected from either the pre-restoration or soil bank treatments.

Soil in each of the three treatment areas was dominated by sand (Table 2). The proportion of silt was highest in soil collected from the pre-restoration treatment and lower in the soil collected from the soil bank and graded areas. The percentage of coarse fragments was highest in the graded treatment and lower in the pre-restoration and soil bank treatment areas.

Only four species of woody vegetation were detected using the Point-Quarter sampling method in the pre-restoration treatment area: *Atriplex lentifomis* (Torr.) S. Watson (quail bush, big saltbrush, big saltbush, quailbrush, lenscale, len-scale saltbush, white thistle), *Baccharis sarothroides* A. Gray(desert broom), *Salix gooddingii* C. R. Ball (willow), and *Tamarix* spp. (tamarisk, salt cedar). Although large cottonwood trees were

Table 1. Characteristics of soil collected at the Tres Rios Ecosystem Restoration and Flood Control Project: soil pH, electrical conductivity (EC) and Munsell Color (n = 3).

Treatment	pH[1]	EC[2] (mS/cm)	Munsell Color
Pre-Restoration	8.12(7.99-8.43)	1.38±0.70 (0.04-2.41)	10yr 5/2 to 10yr 6/3
Soil Bank	7.92(7.76-8.10)	2.03±0.45 (1.15-2.60)	10yr 5/2 to 10yr 7/2
Graded	7.63(7.48-7.76)	4.52±0.96 (3.26-6.41)	10yr 5/2 to 10yr 6/2

[1]Mean pH value and range

[2]Mean EC value, SE and range

Table 2. Characteristics of soil collected at the Tres Rios Ecosystem Restoration and Flood Control Project: percent coarse fragments and texture (n = 3).

Treatment	% Coarse Fragments	% Sand	% Clay	% Silt	Classification
Pre-restoration	1.36	75.24	6.90	17.86	Sandy Loam
Soil bank	5.58	88.23	5.29	6.48	Sand
Graded	20.27	88.32	5.98	5.70	Sand

visible in the pre-restoration area, their density was so low and sporadic that none were detected using the Point-Quarter sampling method. Woody vegetation covered 28.8% of area with the remaining 71.2% bare of woody plant cover. Tamarisk plants were the dominant plants in terms of canopy cover and density followed by quail bush and desert broom (Table 3). Of the four woody species found on the site, only willows are known to form ectomycorrhizal associations. Willows form associations with both AM and EM fungi (Lodge 1989; Lodge and Wentworth 1990; Sasaki et al. 2001). Low levels of colonization by AM fungi have been detected with tamarisk but no association with EM fungi (Beauchamp 2004; Titus et al. 2002). Plants from the genera *Atriplex* and *Baccharis* have been found to form AM but not EM associations (Wang and Qui 2006).

Colonization levels by both AM and EM fungi in roots obtained from soil samples collected from the pre-restoration area were extremely low (ranging from 0.0 to 10.7% with a mean of 0.8% for AM fungi and 1.1% for EM fungi). No living roots could be detected in 10 of the 25 soil samples. Of the 15 living samples assessed, colonization by AM fungi was present in only two samples and colonization by EM fungi in five of the samples.

The percentage of AM colonization in the corn bioassay host was found to be at a moderate levels ranging from 0.0 to 56.0% with the greatest percentage colonization detected in bioassay plants growing in soil collected from the graded treatment (Table 4). AM colonization was detected in the roots of all plants grown in the soil from the soil bank and graded treatments,

Table 3. Woody vegetation in the Tres Rios Ecosystem Restoration and Flood Control Project pre-restoration treatment area.

Species	Density (plants/ha)	Cover (m^2/ha)	Relative Dominance (%)
Atriplex lentiformis	251.6	471.1	16.4
Baccharis sarothroides	35.1	126.2	4.4
Salix gooddingii	58.5	400.5	13.9
Tamarix spp.	239.9	1,877.6	65.3

Table 4. Arbuscular mycorrhizal (AM) infection percentages of bioassay roots (n = 25).

Treatment	AM Colonization (%)[1]	Plants with AM Colonization (%)
Pre-Restoration	17.0±3.2 (0-50)	88.0%
Soil Bank	15.1±3.5 (1-56)	100.0%
Graded	21.6±3.3 (1-53)	100.0%

[1]Mean value, SE and range

but undetected in three plants grown in the soil from the pre-restoration treatment. No significant differences in MIP colonization were detected between treatment areas using ANOVA analysis at the 0.05 confidence level, $F(2,72) = 1.295$, $P = 0.28$ (Table5).

The percentage of EM colonization in the cottonwood bioassay plants was found to be very low (less than 5%) with no significant differences between percentage of colonization in bioassay hosts growing in soil collected from all three areas (Table 6). Colonization by EM fungi could not be detected in the majority of the cottonwood bioassay plants, but there were differences in the percentage of bioassay plants with EM colonization among the treatment areas. The greatest percentage of cottonwood bioassay plants with detectable colonization were grown in soil collected from the pre-restoration area and the lowest percentage were grown in soil collected in the graded area.

Due to low levels of EM colonization of bioassay cottonwood seedlings, roots were collected from living cottonwood and willow trees at the study site to further explore EM presence at the site. EM colonization was detected in roots of all of the cottonwood and willow plants sampled ($n = 10$). The percentage of cottonwood roots with EM colonization ranged from 6.0% to 68.0%, with a mean of 21.1% and SE of 6.4. EM colonization percentages of the willow trees ranged from 1.0% to 72.0% with a mean of 24.5% and SE of 8.0.

Based on the vegetation measurement data, the only vegetation near soil sample points known to form EM associations were willow trees. Willow

Table 5. ANOVA: Mean arbuscular mycorrhizal infection percentages (arcsine transformed) of bioassay plants grown in soil from the three treatment areas (pre-restoration, soil bank, graded).

Source of Variation	Sum of Squares	d.f.	Mean Squares	F	P
Among	0.151	2	0.0754	1.295	0.28
Within	4.190	72	0.0582		
Total	4.340	74			

Table 6. Mycorrhizal infection percentages of EM bioassay roots ($n = 25$).

Treatment	EM Colonization %[1]	% plants with EM Colonization
Pre-Restoration	0.6±.2 (0-4)	28.0%
Soil Bank	0.3±.1 (0-3)	24.0%
Graded	0.2±.1 (0-2)	16.0%

[1]Mean value, SE and range

trees were the closest woody plant to the soil collection point in at least one quadrant for seven of the soil samples. To determine if the presence of willows near the soil sampling point was related to EM colonization of the bioassay plants grown in the corresponding soil, the Fischer's Exact Test of Independence was performed (Table 7). The results of the test determined that the two variables were independent (P = 1). This finding may have been due to the distance between the sampling point and willow; the mean distance from sampling point to willow canopy intercept was 6.7 meters and tree center was 8.1 meters.

Table 7. Fischer's Exact Test of Independence. Variables: EM inoculum present in soil (based on bioassay results) and presence of *Salix gooddingii* (willow) near soil collection point.

		EM inoculum present in soil	
		Yes	No
Willlow near collection point	Yes	2	5
	No	5	13

2-Tail : P-value =1

CHAPTER 4

DISCUSSION

In this restoration project, extensive vegetation removal and soil disturbance took place. The upper 46 cm of soil in this tamarisk affected riparian area was removed followed by chipping of the removed vegetation, soil banking and grading. The project provided an opportunity to examine the impact of these restoration activities on propagules/inoculum of mycorrhizal fungi and to evaluate the need to inoculate with AM or EM fungi when re-vegetating the site. Because plant removal and soil disturbance can be associated with decreases in mycorrhizal fungal propagules (Galvez et al. 2001; Borie et al. 2006; Li et al. 2007; Celik et al. 2011), it was hypothesized that propagule levels of mycorrhizal fungi would be lower in soils collected from the soil banks and graded areas than in soil collected from the riparian areas unaffected by restoration activities.

Contrary to expectations, inoculum of both AM and EM fungi were found to be at similar levels in all three treatment areas, which differed in both soil disturbance (pre-restoration, soil bank, graded) and soil properties (minor differences in pH, EC, texture, percent coarse fragments). The finding of AM fungal tolerance to mechanical soil disturbance is similar to the results of Richter et al. (2002) who found AM inoculum levels to be higher in a riparian field that was disked every 3 to 4 years in comparison to a neighboring grassland and Urcelay et al. (2009) who found that AM fungal inoculum was reduced five months after plant removal but returned to the level of control plots within 17 months.

AM fungal inoculum was detected at moderate levels in all three treatment areas in contrast to EM fungal inoculum which was detected in very low levels in each area. Differences in the levels of EM and AM fungal propagules at this site may be related to availability of host plants. The results of the vegetation measurements confirmed that the site is dominated by tamarisk. Cottonwood and willows, which are native riparian host plants for EM fungi, were present on the site but in low density and low percentage of canopy cover. Tamarisk and other non-EM shrubs accounted for 86.1% of the woody vegetation cover, willow 13.9%. Cottonwoods were present on the site but their density was so low and sporadic that none were detected by the sampling methodology used. Colonization of established cottonwood and willow trees by EM fungi at the site indicates that EM fungi were present in this riparian area but propagules were detected in very low amounts in soil away from those plants. The moderate levels of AM fungal propagules in all soil treatments could be due to the greater presence of host plants for AM fungi as many desert riparian forbs and grasses have been found to form AM associations (Kennedy et al. 2002; Beauchamp 2004).

These findings are consistent with prior research indicating that AM fungi are less affected by tamarisk invasions than EM fungi (Johnson, 2005; Meinhardt and Gehring, 2012). Johnson (2005) studied mycorrhizal root colonization and community structure at eight sites along the San Pedro River in Arizona and found that EM propagule levels were lower and AM propagule levels higher at a site dominated by tamarisk. Meinhardt and Gehring (2012) found that the presence of tamarisk had a greater negative

impact on EM than AM symbioses. In their study, EM propagule abundance in the soil beneath tamarisk was reduced and community composition altered. However, there were no significant changes in AM fungal spore communities or propagule abundance. In their bioassay studies, there was no difference in colonization rates for AM bioassay bait plants (corn) grown in soil from sampled under tamarisk versus soil sampled under cottonwoods. However, EM colonization of bait plants (ponderosa pine) grown in soil from under tamarisk was significantly lower than those grown in soil from under cottonwoods.

Meinhard and Gehring (2012) hypothesized that the larger negative effect of tamarisk on EM fungi than AM fungi may have been due to increased sensitivity of EM fungi to the changes in soil chemistry. They found that nitrate concentration and soil salinity were increased in the presence of tamarisk. Previous studies have found AM fungi to have a greater tolerance for environmental stressors such as high and low soil moisture (Lodge 1989) and salt stress (Giri and Mukerji 2004).

The roots found in soil from the pre-restoration area were found to have low levels of AM colonization. However, AM propagules, as assessed through growth of bioassay plants, were available in moderate levels. Colonization of existing roots may have been low for a variety of reasons such as time of year, amount of water, and whether the roots were from plants that form AM associations. The bioassay measures the number of propagules (spores, actively growing hyphae, and dormant pieces of hyphae) in the soil that are available to infect plants under ideal conditions. In this study, the

bioassay was conducted with a host that supports high levels of AM colonization and was grown under well watered conditions.

Implications for Practice

A moderate level of AM inoculum potential was found throughout the stages of this project, indicating a tolerance by AM fungi for tamarisk presence and for the soil disturbance associated with the restoration activities. In projects such as the Tres Rios Ecosystem Restoration and Flood Control Project in which tamarisk dominated riparian areas are being restored and tamarisk removed, it does not appear necessary to inoculate soil with AM fungi prior to planting native vegetation.

When planting trees that form EM associations (e.g. cottonwoods and willows), it may be beneficial to augment soil or pre-inoculate plants with EM fungi prior to planting. EM inoculum levels in this tamarisk dominated site were low, even prior to restoration. Local riparian areas that have native cottonwoods and willows but fewer tamarisk are likely to be a better source of EM inoculum.

LITERATURE CITED

Allen, M.F. 1987. Re-establishment of mycorrhizas on Mount St. Helens: Migration vectors. Trans. British Mycology Soc. 88:413-417.

Beauchamp, V.B., J.C. Stromberg, and J.C. Stutz. 2005 Interactions between *Tamarix ramosissima* (saltcedar), *Populus fremontii* (cottonwood) and mycorrhizal fungi: Effects on seedling growth and plant species coexistence. Plant Soil 275:221-231.

Beauchamp, V.B., J.C. Stromberg, and J.C. Stutz. 2006. Arbuscular mycorrhizal fungi associated with *Populus-Salix* stands in a semi-arid riparian ecosystem. New Phytologist 170:369-380.

Borie, F., R. Rubio, J.L. Rouanet, A. Morales, G. Borie, and C. Rojas. 2006. Effects of tillage systems on soil characteristics , glomalin, and mycorrhizal propagules in a Chilean Ultisol. Soil and Tillage Research 88:253-261.

Brower, J.E., J.H. Zar J.H., and C.N. von Ende. 1998. Field and Laboratory Methods for General Ecology, 4th Edition. WCB McGraw-Hill, pages 103-109.

Celik, I., Z.B. Barut, I. Ortas, M. Gok, A. Demirbas, Y. Tulun, and C. Akpinar. 2011. Impacts of different tillage practices on some soil microbiological processes and crop yield under semi-arid Mediterranean conditions. International Journal of Plant Production 5:237-254.

City of Phoenix, 2012. Tres Rios Full Scale Wetlands Project. http://phoenix.gov/TRESRIOS/future.html

Galvez, L., D.D. Douds, L.E. Drinkwater, and P. Wagoner. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant Soil 228:299-308.

Giovanetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84:489-500.

Govindarajula, M., P.E. Pfeffer, H. Jin, J. Abubaker, D. D. Douds, J.W. Allen, H. Bücking, P.J. Lammers, and Y. Shacher-Hill. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819-823.

Giri, B. and K. G. Mukerji. 2004. Mycorrhizal inoculants alleviate salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14:307–312.

Johnson, C.N. 1996. Interactions between mammals and ectomycorrhizal fungi. Trends in Ecology and Evolution. 11:503-507.

Johnson, J.D. 2005. Mycorrhizal fungal associations with Populus fremontii and Tamarix ramosissima on the San Pedro River, Arizona. Master's thesis, Arizona State University.

Kennedy, L.J., R.L. Tiller, and J.C. Stutz. 2002. Associations between arbuscular mycorrhizal fungi and *Sporobolus wrightii* in riparian habitats in arid southwestern North America. Journal of Arid Environments 50:459-475.

Klironomos, J.N., and M. M. Hart. 2002. Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12:181–184.

Koske, R.E., and J.N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizae. Mycological Research 92:486–488.

Li, L.F., T. Li, and Z.W. Zhao. 2007. Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never cultivated field in hot and arid ecosystem of southwest China. Mycorrhiza 17:655-665.

Lodge, D.J., 1989. The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in Populus and Salix. Plant and Soil 117:243-253.

Lodge, D.J. and T.R.Wentworth. 1990. Negative associations among VA-mycorrhizal fungi and some ectomycorrhizal fungi inhabiting the same root system. Oikos 57:347-356.

McDonald, J.H. 2009. Handbook of Biological Statistics, 2nd ed. Sparky House Publishing, Baltimore, Maryland. http://udel.edu/~mcdonald/statintro.html

Meinhardt, K.A., and C.A. Gehring. 2012. Disrupting mycorrhizal mutualisms: A potential mechanism by which exotic tamarisk outcompetes native cottonwoods. Ecological Applications 22:532–549.

Natural Resources Conservation Service (NRCS). 1993. Soil survey manual. Soil Conservation Service. U.S. Department of Agriculture Handbook 18. http://soils.usda.gov/technical/manual/contents/chapter3.html.

Newman, Mike (personal communication), Kiewit Corporation.

Richter , B.S., R. L. Tiller, and J.C. Stutz. 2002. Assessment of arbuscular mycorrhizal fungal propagules and colonization from abandoned agricultural fields and semi-arid grasslands in riparian floodplains. Applied Soil Ecology 20:227–238.

Sasaki, A., M. Fujiyoshi, S. Shidara, and T. Nakatsubo. 2001. Effects of nutrients and arbuscular mycorrhizal colonization on the growth of *Salix gracilistyla* seedlings in a nutrient-poor fluvial bar. Ecological Restoration. 16:165-172.

Schnoor, T. K., Y. Lekberg, S. Rosendahl, and P.A. Olsson. 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. Mycorrhiza 21:211–220.

Schussler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the Glomeromycota, phylogeny and evolution. Mycological Research 105:1413-1421.

Shafroth, P.B., J.R. Cleverly, T.L. Dudley, J.P. Taylor, C. van Riper III, E.P. Weeks, and J.N. Stuart. 2005. Control of *Tamarix* in the western United States: Implications for water salvage, wildlife use, and riparian restoration. Environmental Management, 35: 231-246.

Shah, J.J.F., C.N. Dahm, S.P. Gloss, and E.S. Bernardt. 2007. River and Riparian Restoration in the southwest: Results of the National River Restoration Science Synthesis Project. Restoration Ecology 15:550–562.

Smith, S.E., and D.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press.

Stutz, J.C., V.B. Beauchamp, J. Johnson, L.J. Kennedy, B.S. Richter, and K.M. Jacobson. 2009. Mycorrhizal ecology. Pages 73-88 in J.C. Stromberg and B. Tellman, eds. Ecology and Conservation of the San Pedro River. University of Arizona Press.

Titus, J.H., P.J. Titus, R.S. Nowak, and S.D. Smith. 2002. Arbuscular mycorrhizae of Mojave Desert plants. Western North American Naturalist 62: 327-334.

Trappe, J.M. 1987. Phylogenetic and ecological aspects of mycotrophy in angiosperms from an evolutionary standpoint. Pages 2-25 in F.R. Safir, ed. Ecophysiology of VA Mycorrhizal Plants, CRC Press.

Urcelay, C., S. Diaz, D.E. Gurvich, F.S. Chapin III, E. Cuevas, and L.S. Domınguez. 2009. Mycorrhizal community resilience in response to experimental plant functional type removals in a woody ecosystem. Journal of Ecology 97:1291–1301.

Wang, B., and Y L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16: 299–363.

Warner, N.J., M. F. Allen, and J. MacMahon. 1987. Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. Mycologia 79:721-730.